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| EXAMINER | |
|---|---|
| HILL, KEVIN KAI | |

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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/642,255 | DOLE ET AL. | |
| | Examiner | Art Unit | |
| | Kevin K. Hill, Ph.D. | 1633 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *30 July 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,5-9,12-15,18-20,22,26 and 31-42* is/are pending in the application.

4a) Of the above claim(s) *31 and 37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5-9,12-15,18-20,22,26,32-36 and 38-42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 August 2003* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## Detailed Action

Applicant has elected with traverse the invention of Group II, Claims 1-30, 32-36 and 38-41, drawn to an *in vivo* gene therapy method for treating critical limb ischemia (CLI). Claims 31 and 37 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Within Group I, Applicant has elected the eNOS polypeptide species to comprise a first mutation at a position corresponding to amino acid 495 and a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO:1, as recited in Claim 7.

However, the species election regarding the eNOS polypeptides comprising the first, second and third mutations has been withdrawn because those sequences are free of the prior art. Applicant may access the results of the sequence searches in SCORE.

Within Group I, Applicant has elected the angiogenic factor species "FGF", as recited in Claim 30.

## *Amendments*

Applicant's response and amendments, filed July 30, 2007, to the prior Office Action is acknowledged. Applicant has cancelled Claims 3-4 and 27-30, withdrawn Claims 31 and 37, amended Claims 1, 5-9, 12-15, 18-20, 22, 26, 32-36 and 38-41, and added new claims, Claim 42. Applicant's new claims have been entered into the application as requested and will be examined on the merits herein, as they are considered to belong to the elected group.

Claims 31 and 37 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Claims 1, 5-9, 12-15, 18-20, 22, 26, 32-36 and 38-42 are under consideration.

## *Priority*

Applicant's claim for the benefit of a prior-filed application, parent provisional application 60/403,637, filed on August 16, 2003 under 35 U.S.C. 119(e) is acknowledged. Accordingly, the effective priority date of the instant application is granted as August 16, 2003.

## *Drawings*

1. **New corrected drawings in compliance with 37 CFR 1.121(d) are required** in this application because Figure 25 (page 25/28) is entitled as "Figure 7"; however, the title "Figure 7" is originally used in the figure on page 7/28. Applicant is strongly encouraged to review each figure for correctness in charting, graphing, data presentation and proper titles.

Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the U.S. Patent and Trademark Office no longer prepares new drawings. The

corrected drawings are required in reply to the Office action to avoid abandonment of the application. The requirement for corrected drawings will not be held in abeyance.

***Response to Amendment***

The Examiner appreciates Applicant's explanation regarding how the data is graphed in the drawings.

While Applicant purports to have provided a copy of Duan et al (2000) and correction of the mis-numbering of Figure 25 in the papers filed July 30, 2007, the Examiner is unable to find record of said papers actually having been filed.

## *Claim Objections*

2. **The prior objections to Claims 1 and 35-36 is withdrawn** because Applicant has amended the claims to first identify the polypeptide by its complete name prior to using its acronym

3. **Claims 1 and 20 are objected to because of the following informalities**:

With respect to claim 1, a space should be placed between the terms "polypeptide" and "comprises" recited on line 4. Furthermore, it appears that the word "as" immediately following the above-mentioned term "comprises" may contain a typographical error for the word "at".

With respect to claim 20, Applicant is advised that should claim 7 be found allowable, claim 20 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Appropriate correction is required.

## *Claim Rejections - 35 USC § 112*

4. **The prior rejection of Claims 1-30, 32-36 and 38-41 under 35 U.S.C. 112, first paragraph, written description, is withdrawn** in light of applicant's argument that the sequences disclosed in the specification are already known, including recognized structure/function relationships between the recited genes and their corresponding proteins/domains for humans, as well as other mammalian species.

5. **The prior rejection of Claims 1-30, 32-36 and 38-41 under 35 U.S.C. 112, first paragraph, is withdrawn** in light of Applicant's amendments to the claims emphasizing that the eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wildtype eNOS in mammalian cells, said calmodulin-biding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1, in addition to arguments showing support in the specification regarding how to produce and select the requisite polypeptides for use in the claimed method.

## *Claim Rejections - 35 USC § 112*

6. **The prior rejections of Claims 12-21 under 35 U.S.C. 112, second paragraph, is withdrawn** in light of Applicant's amendments to the claims to identify the referenced eNOS polypeptide as being a wildtype eNOS polypeptide of SEQ ID NO:1.

## *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. **The prior rejection of Claims 1-2, 4, 6, 10, 12-13, 15-18, 20-26, 32, 34-36 and 39 under 35 U.S.C. 103(a)** is moot in light of Applicant's amendments to the base claims 1 and 35-36.

8. **Claims 1-2, 5-7, 9-10, 12-26, 32-36 and 38-40 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Smith et al (*of record in IDS), Fleming et al (*of record in IDS) and Fulton et al (*of record in IDS).

The claims are drawn to a method of treating critical limb ischemia comprising administering to a patient in need of treatment an effective amount of a polynucleotide encoding a mammalian endothelial nitrogen oxide synthase (eNOS) polypeptide comprising at least one

mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wildtype eNOS in mammalian cells, said calmodulin-biding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1.

Smith et al teach a method of administering to a rat hindlimb ischemic model a polynucleotide encoding a human wildtype eNOS polypeptide (pg 1280, column 2, "Femoral Artery Ligation"; pg 1281, column 2, "Time Course of Human eNOS Expression"). The polynucleotide encoding the human eNOS was contained in an adenoviral vector operably linked to a cytomegalovirus (CMV) promoter (pg 1279, column 2, lines 14-17; pg 1280, column 1, "Construction of eNOS Adenovirus"). The adenoviral vector is administered by intramuscular injection, wherein eNOS activity is thus modulated in the cells of said patient (pg 1281-1284, Results).

Smith et al do not teach a mutant mammalian eNOS polypeptide comprising at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wildtype eNOS in mammalian cells, said calmodulin-biding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1. However, at the time of the invention, Fleming et al taught that the dual phosphorylation of Ser1177 and Thr495 determines the activity of eNOS (pg 1, Abstract), wherein Thr495 corresponds to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wildtype eNOS in mammalian cells, said calmodulin-biding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1. Fleming et al teach that the non-phosphorylatable Alanine mutation (T495A) (thereby decreasing the phosphorylation as compared to wildtype eNOS polypeptide) allows the calmodulin-binding domain to continue association with calmodulin and retains enzymatic activity, inherently possessing an increased binding affinity for calmodulin, decreased calcium dependence in calcium-calmodulin-mediated stimulation and increased eNOS activity, as compared to wildtype eNOS (pg 5, col. 2, ¶1; pg 6, col. 1 and Figure 9B). The association of calmodulin with eNOS initiates NO production, and is regulated by the calcium-dependent dephosphorylation of eNOS Thr495. Thus, the non-phosphorylatable T495A mutation that permits association of calmodulin with eNOS inherently possesses the NO generation activity.

Neither Smith et al nor Fleming et al teach a mutant mammalian eNOS polypeptide comprising a mutation corresponding to amino acid 1177 of SEQ ID NO:1. However, at the time of the invention, Fulton et al taught a mutant mammalian eNOS polypeptide in which the amino acid residue corresponding to amino acid residue 1177 of SEQ ID NO:1 is substituted to Aspartate (S1177D) resulting in a gain-of-function enzyme (pg 599, Figure 3 and legend). Smith et al note that the S1179 of the instant eNOS corresponds to S1177 of human eNOS (pg 599, column 2, lines 1-3), and thus also corresponds to amino acid residue 1177 of SEQ ID NO:1. Fulton et al teach that phosphorylation of S1177 is functionally important for NO release, and thus permits activated calmodulin-binding at lower calcium concentrations (pg 600, column 1, line 22; column 2, lines 20-22). Furthermore, Fulton et al suggest that regulation of such phosphorylation may provide a novel therapeutic target for the design of drugs aimed at improving endothelial function in cardiovascular disease associated with dysfunction in the synthesis or biological activity of NO (pg 600, column 2, conclusory sentence).

It would have been obvious to one of ordinary skill in the art to modify the adenoviral vector encoding the human eNOS polypeptide of Smith et al to encode an eNOS polypeptide comprising T495A and S1177D mutations with a reasonable chance of success because the art teaches that the S1177D and T495A mutations result in enzymes with greater activity. All the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. An artisan would have been motivated to introduce T495A and S1177D mutations into a wildtype eNOS polypeptide because Fleming et al suggest that maximal activation of eNOS at physiological concentrations of calcium and calmodulin requires the simultaneous phosphorylation of Ser1177 and dephosphorylation of Thr495 (pg 7, col. 2, lines 4-8), wherein said double mutant eNOS would have increased reductase and NO generation activities, as compared to wildtype eNOS. Wildtype eNOS requires post-translational modifications, specifically phosphorylation of particular amino acid residues including 495 and 1177, that regulates and limits the activity of the normal enzyme; whereas, the T495A mutation and the S1177D mutation mimic the activating post-translational modifications, obviating the requirement for regulated activation, decreasing sensitivity to NOS inhibitors, and resulting in an

enzyme *a priori* with as much activity as a fully activated wildtype eNOS polypeptide. Furthermore, Fulton et al suggest that drugs that promote the phosphorylation of eNOS, and thereby activating eNOS, would be of therapeutic value. Given that both T495A and S1177D mutations result in gain-of-function eNOS enzymes, it would be more efficient and advantageous to use the existing mutations in the artisan's desired eNOS polypeptide rather than having the artisan expend resources to identify a drug with that will promote the same enzymatic activity, though more indirectly, as the instant mutations.

Thus, the invention as a whole is *prima facie* obvious.

9. **Claims 1, 9, 35-36, 38, 40 and 42 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Smith et al (*of record in IDS), Fulton et al (*of record in IDS) and Fleming et al (*of record in IDS), as applied to claims 1-2, 5-7, 9-10, 12-26, 32-36 and 38-40 above, and in further view of Alberts et al (*Molecular Biology of the Cell*, Third Edition, Garland Publishing, New York, New York, 1994).

The prior cited art does not teach a Thr4955 mutation to be Valine, Leucine or Isoleucine. However, the art recognizes (Alberts et al) that Alanine, Valine, Leucine or Isoleucine each possess non-polar side chains and are uncharged amino acids. Absent evidence to the contrary, nothing non-obvious is seen with substituting Alanine for the Valine, Leucine or Isoleucine because each is an art-recognized conservative substitution and each would result in the same functional consequence of the eNOS polypeptide, specifically achieving a non-phosphorylatable amino acid residue in the calmodulin-binding domain, thereby resulting in a polypeptide having increased eNOS activity. A simple substitution of one known, equivalent element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

Thus, the invention as a whole is *prima facie* obvious.

10. **Claims 1, 8-11, 35-36 and 41-42 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Smith et al (*of record in IDS), Fulton et al (*of record in IDS), Fleming et al

(*of record in IDS) and Alberts et al (*Molecular Biology of the Cell*, Third Edition, Garland Publishing, New York, New York, 1994) as applied to claims 1-2, 5-7, 9-10, 12-26, 32-36, 38-40 and 42 above, and in further view of Liu et al (J. Cell Biol. 137(7): 1525-1535, 1997).

The prior cited art does not teach the use of an eNOS polypeptide comprising a mutation at a position corresponding to amino acid 2 of SEQ ID NO:1. However, at the time of the invention, Liu et al taught that eNOS is a dually acylated peripheral membrane protein that targets into the Golgi region and plasma membrane, and compartmentalization is required for efficient production of NO in response to agonist challenge. Liu et al demonstrated that G2A substitution are known in the art to abrogate myristoylation, and that in the context of eNOS mutation of the N-myristoylation site inhibits palmitoylation and randomly distributes eNOS into the cytosol (pg 1532, col. 2, last 4 lines).

It would have been obvious to one of ordinary skill in the art to modify an eNOS polypeptide comprising a T495A mutation, alone or in combination with a S1177D mutation, with a G2A mutation with a reasonable chance of success because the particular technique of G2A mutation to abrogate N-myristoylation of eNOS was recognized as part of the ordinary capabilities of one skilled in the art. The art recognizes that soluble isoforms of NO synthase exist, e.g. nNOS and iNOS, and Liu et al taught that G2A mutation renders the eNOS polypeptide similarly soluble. An artisan would have been motivated to combine a G2A mutation with the aforementioned mutations because membrane association of eNOS places the polypeptide into close proximity of negative regulatory proteins. A soluble, mutant eNOS polypeptide that does not require regulated activation would be more efficacious for not being as susceptible to negative regulation as an eNOS polypeptide tethered to the membrane.

Thus, the invention as a whole is *prima facie* obvious.

## *Conclusion*

11. No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Kevin K. Hill

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